JOURNAL OF CLINICAL MICROBIOLOGY, May 1979, p. 565-569
0095-1137/79/05-0565/05$02.00/0

Vol. 9, No. 5

Evaluation of the Modified API 20C System for Identification of Clinically Important Yeasts

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Received for publication 9 February 1979

The modified API 20C system (Analytab Products, Inc.) containing 19 carbohydrate assimilation tests was used to identify stock cultures of clinical isolates and routine clinical isolates from the Mayo Clinic mycology laboratory. The system provided correct identifications for 96% of the 505 organisms tested. The API 20C represents a commercial system useful for the identification of yeasts from clinical specimens. Although reliable, it is not a complete system and must be used in conjunction with microscopic morphological features for definitive identification. Since the system requires 72 h for identification, it is not designed for the rapid presumptive identification of such organisms as *Cryptococcus neoformans*; other biochemical tests must be used for this purpose.

In recent years, the number of serious yeast infections, particularly in the immunocompromised patient (5), has increased significantly. This situation has necessitated that clinical laboratories become more proficient in their ability to isolate and rapidly identify yeasts of medical importance. Traditional procedures for yeast identification (9) are considered by many to be technically complex and time consuming. Also, many reagents required for identification of the yeasts to species are not commercially available. In 1975, the API 20C microtube system, a commercial product (Analytab Products, Inc.) for the identification of yeasts, was introduced. This system miniaturized the traditional procedures of carbohydrate fermentations and assimilations, permitting identification of yeasts within 72 h after inoculation (2, 8, 11). Recently, this system has undergone extensive modification. The carbohydrate fermentation tests have been eliminated and replaced by additional assimilation tests. In addition, a numerical approach with a computer-assisted system for the identification of only clinically important yeasts has been introduced. It was the purpose of this study to determine whether the modified API 20C system provides a reliable and accurate method for the identification of medically important yeasts.

MATERIALS AND METHODS

Since the API 20C system was developed to provide identifications for only clinically important yeasts, 505 isolates of yeasts and yeastlike organisms recovered from clinical specimens were used in this study. Two hundred fifty-one isolates were selected from the Mayo Clinic stock culture collection and coded so that the identity was unknown until all data were analyzed. The remaining 254 isolates were recovered from respiratory secretions by the Mayo Clinic mycology laboratory and all were identified within 2 to 10 days. The identification of each organism was made using methods previously described by Roberts (6) and Koneman et al. (4). All organisms used in the study were identified by the conventional method used in this laboratory and by the API 20C yeast system so that a comparison could be made between the identifications given by both methods. No attempt was made to compare the methods on a substrate to substrate basis.

Conventional method. The conventional method used by the Mayo Clinic mycology laboratory is described in detail by Roberts (6) and by Koneman et al. (4). Identifications were based on germ tube formation, microscopic morphology on cornmeal-Tween 80 agar, urease production, nitrate reduction, temperature tolerance, pigment production on niger seed agar, the caffeic acid-ferric citrate reaction, and carbohydrate assimilation and fermentation.

An auxanographic method (4) was used to determine assimilation of glucose, maltose, sucrose, lactose, raffinose, trehalose, galactose, melibiose, and inositol. A suspension of yeast in sterile saline equivalent to a McFarland standard no. 4 was used to flood the surface of a 100- by 15-mm petri dish containing yeast nitrogen base (Difco) and 0.4% bromocresol purple (Difco). Carbohydrate disks (Difco) 6 or 13 mm in diameter were spaced evenly on the agar surface. The plates were incubated at 30°C for 24 to 48 h and examined for a color change from purple to yellow or for growth around the disks, which indicated assimilation of the carbohydrate substrates.

Fermentation of glucose, maltose, sucrose, and lactose was determined by inoculating carbohydrate fermentation tubes (Difco) with 5 drops (0.2 ml) of the yeast-saline suspension. The tubes were incubated at 37°C and read after 24 and 48 h and again after 10 days for the presence of gas in the inverted Durham tubes.

API 20C method. All tests were performed according to the manufacturer's instructions. Kits were stored at 4°C until used. Ampoules containing the API 20C basal medium were placed in a boiling-water bath and allowed to remain for 5 min after melting to ensure complete liquification. Each ampoule was removed to a 50°C water bath and held there until inoculation. Once melted, the ampoules were used within 2 h. The inoculum was prepared by lightly touching an isolated yeast colony with the tip of a sterile wooden applicator stick and then grinding the tip against the bottom of an ampoule containing the molten basal medium. A uniform suspension was achieved by gentle stirring, with care being taken to avoid the excessive production of air bubbles. The density of the inoculum was adjusted to just below 1+ when held against a Wickerham card. With a sterile Pasteur pipette, the suspension was inoculated into each of the 20 plastic cupules on the API strip. The first cupule contained only basal medium and served as a zero-growth control. The remaining cupules contained dehydrated substrates used to detect assimilation of the carbohydrates glucose, glycerol, 2-keto-D-gluconate, L-arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, methyl-D-glucoside, *N*-acetyl-D-glucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, and raffinose. The strips containing the cupules were placed in plastic trays containing 5.0 ml of water and were incubated at 30°C for 72 h. Readings were made after 24, 48, and 72 h of incubation. Turbidity greater than that of the growth control was used as an indication of assimilation. After the 72-h reading, a seven-digit profile number based on the organism's assimilation reactions was obtained according to instructions provided by the manufacturer. The identification of organisms was based on the numerical Profile Index enclosed with the commercial product in conjunction with the microscopic morphology on cornmeal-Tween 80 agar (4). When a profile number was encountered which was not listed in the Profile Index, the API computer service was consulted.

Criteria used for the identification of organisms by the modified API 20C system were as follows. (i) A correct identification was obtained if a seven-digit profile number obtained from the assimilation reactions of an organism was listed in the Profile Index supplied by the manufacturer. (ii) A correct presumptive identification was obtained if an assimilation profile number of an organism was not listed in the Profile Index and the identification required consultation of the API computer service. In this instance the computer lists three possible choices, in decreasing order of likelihood, for the identification of an unknown organism. If the first choice listed was correct and described as an excellent, very good, or acceptable identification, the identification was considered as presumptively correct. If the correct identification was listed among the three choices and could be differentiated on the basis of morphology on cornmeal-Tween 80 agar, the identification was also considered as presumptively correct.

One hundred forty stock culture isolates of yeast and yeastlike organisms were subcultured onto Emmon's modification of Sabouraud dextrose agar and inhibitory mold agar containing 5 μg of gentamicin and 125 μg of chloramphenicol per ml. The API 20C yeast system was inoculated with organisms from each medium to determine the effect of antibiotics on the final identification of the organisms tested.

RESULTS

The modified API 20C system provided correct identifications for 486 or 96% of the 505 isolates tested (correct plus presumptively correct identifications; incorrect identifications, 19 or 4%). Of the 251 stock cultures tested, 236 or 94.1% were identified correctly, including 25 (10%) which required consultation of the API computer service, whereas 15 (5.9%) were incorrectly identified (Table 1).

The modified API 20C system provided correct identifications for 98.4% of the 254 fresh clinical isolates examined, including 19 (7.5%) which required consultation of the API computer service (Table 2), whereas 4 (1.6%) were incorrectly identified.

The conventional method provided correct identifications for all fresh clinical isolates with one exception, a weakly pigmented strain of *Rhodotorula*.

One hundred forty-two organisms maintained as stock cultures were subcultured onto both Sabouraud dextrose agar (Emmon's modification) and inhibitory mold agar containing 5 μg of gentamicin and 125 μg of chloramphenicol per ml before inoculation of the API 20C strips. The modified API system provided correct identifications for 95% of the organisms tested from both media, which suggested that the identifications were not influenced by the type of medium used before testing.

The optimal time required for the identification of the organisms tested was 72 h. However, 82.5% of the *Torulopsis glabrata* isolates were identified after 24 h, and identification of all isolates of this species was complete after 48 h. Two of seven isolates of *Cryptococcus terreus* required incubation for 96 h before identification was made due to delayed inositol assimilation reactions. A majority of the cryptococci tested required 72 h of incubation before assimilation of inositol could be detected.

Table 3 lists the organisms misidentified by the modified API system and the reasons for misidentification. At present, the assimilation patterns for *Candida lambica* and *Cryptococcus luteolus* are not included in the API computer data base and, therefore, cannot be identified by this system. The most frequently encountered problem involved the identification of *Candida albicans* and *Candida tropicalis*. Three isolates of *C. albicans* were identified by the API 20C system as *C. tropicalis* due to the false-positive assimilation of melezitose. One *C. tropicalis* iso-

TABLE 1. *Identification of stock cultures of clinical isolates by the modified API 20C*

Organism	No. tested	Modified API 20C		
		No. correct (%)	No. correct, presumptive (%)	No. incorrect (%)
Candida albicans	21	18 (85.7)	2 (9.5)	1 (4.8)
C. guilliermondii	9	7 (77.8)	1 (11.1)	1 (11.1)
C. krusei	20	19 (95)	1 (5)	0
C. lambica[a]	1	0	0	1 (100)
C. parapsilosis	25	22 (88)	2 (8)	1 (4)
C. pseudotropicalis	10	10 (100)	0	0
C. rugosa	1	0	1 (100)	0
C. tropicalis	24	16 (66.7)	7 (29.2)	1 (4.1)
Cryptococcus albidus	24	23 (95.8)	1 (4.2)	0
C. laurentii	9	9 (100)	0	0
C. luteolus[a]	5	0	0	5 (100)
C. neoformans	38	33 (86.8)	2 (5.2)	3 (8)
C. terreus	7	4 (57.1)	1 (14.3)	2 (28.6)
C. uniguttulatus	3	3 (100)	0	0
Geotrichum candidum	1	1 (100)	0	0
Rhodotorula glutinis	7	0	7 (100)	0
R. pilimanae	2	0	2 (100)	0
R. rubra	1	0	1 (100)	0
Saccharomyces cerevisiae	10	10 (100)	0	0
Torulopsis glabrata	22	22 (100)	0	0
Trichosporon beigelii	11	6 (54.5)	5 (45.5)	0
Total	251	211 (84.1)	25 (10)	15 (5.9)

[a] Presently not included in computer data base of modified API 20C system.

TABLE 2. *Identification of fresh clinical isolates by the modified API 20C*

Organism	No. tested	No. correct (%)	No. correct, presumptive (%)	No. incorrect (%)
Candida albicans	28	24 (85.7)	2 (7.15)	2 (7.15)
C. guilliermondii	5	5 (100)	0	0
C. krusei	13	13 (100)	0	0
C. parapsilosis	37	30 (81.1)	7 (18.9)	0
C. pseudotropicalis	3	3 (100)	0	0
C. tropicalis	34	34 (100)	0	0
C. zeylanoides	2	2 (100)	0	0
Cryptococcus albidus	15	12 (80)	3 (20)	0
C. laurentii	1	0	1 (100)	0
C. luteolus	1	0	0	1 (100)
C. neuformans	16	14 (87.5)	2 (12.5)	0
Rhodotorula sp.	22	17 (77.3)	4 (18.2)	1 (4.5)
Saccharomyces cerevisiae	9	9 (100)	0	0
Torulopsis glabrata	64	64 (100)	0	0
Trichosporon beigelii	4	4 (100)	0	0
Total	254	231 (90.9)	19 (7.5)	4 (1.6)

late was identified as *C. albicans* because of a false-negative melezitose reaction.

DISCUSSION

The modified API 20C system provided correct identifications for 96% of the organisms tested. This is comparable to the accuracy of the original system as determined in previous studies (2, 8, 11).

The modified API 20C system required much less technical proficiency than did the original system. With the elimination of carbohydrate fermentation tests, dehydrated substrate is now contained in 20 plastic cupules rather than in tubules. This modification has simplified filling with the agar-inoculum suspension and has eliminated concern over entrapped air spaces and the use of Vaspar seals.

TABLE 3. *Analysis of incorrectly identified organisms by the API 20C system*

Organism	No.	Explanation of incorrect identification
Candida albicans	3	Identified as *C. tropicalis* due to positive assimilation of melezitose
C. guilliermondii	1	Arabinose, xylose, xylitol, and melezitose not assimilated after 72 h
C. lambica	1	Profile not included in computer data base
C. parapsilosis	1	Arabinose not assimilated after 72 h; false-positive assimilation of xylitol and cellobiose
C. tropicalis	1	Identified as *C. albicans* due to negative assimilation of melezitose
Cryptococcus luteolus	6	Profile not included in computer data base
C. neoformans	3	One did not assimilate adonitol, *N*-acetyl-D-glucosamine, trehalose, or raffinose after 72 h; one gave false-positive assimilation of glycerol; one did not assimilate inositol, xylitol, arabinose, or raffinose after 72 h.
Rhodotorula sp.	1	Did not assimilate sucrose, maltose, melezitose, or raffinose after 72 h.

The proper inoculation density was of some importance. A too dense inoculum resulted in apparent false-positive assimilation reactions and in a "carryover" phenomenon where growth was observed in the zero-growth control cupule. This should provide little problem if the inoculum is adjusted to the proper density by use of the Wickerham card and if the reader compares the individual carbohydrate assimilation tests with the zero-growth control cupule.

The manufacturer recommends that the API strips be incubated at 30°C and read after 24, 48, and 72 h. These instructions are appropriate and should be followed since most cryptococci studied required 72 h for identification. If the reactions appear incomplete after 72 h and microscopic morphology resembles *Cryptococcus*, we recommend that the strips be held at 30°C for an additional 24 h. Some strains of cryptococci may require the additional incubation time before inositol assimilation can be detected.

In the instructions, the manufacturer states that well-isolated colonies on Sabouraud dextrose agar serve as the inoculum source. Our results indicate that the inoculum may also be prepared from organisms grown on inhibitory mold agar which contains chloramphenicol (125 μg/ml) and gentamicin (5 μg/ml). The presence of these antibiotics did not affect the assimilation patterns or the final identifications of the organisms tested. However, antibiotic-containing media should be used with caution. API strips inoculated with yeast taken from selective media are subject to contamination due to the presence of bacteria whose growth was suppressed by the antibiotics in the media.

The identification of yeasts based solely on the assimilation patterns provided by the modified API 20C system is not reliable unless used in combination with microscopic morphological features (presence of arthroconidia, blastoconidia, hyphae, or pseudohyphae) on cornmeal-Tween 80 agar as recommended by the manufacturer. *Crytococcus laurentii*, *C. terreus*, and *Candida guilliermondii* can give an assimilation pattern identical to that of *Trichosporon beigelii*. In addition, the following pairs of organisms can exhibit identical assimilation reactions: *Trichosporon capitatum* and *Candida krusei*, *Torulopsis maris* and *Candida rugosa*, *T. maris* and *Trichosporon penicillatum*, and *Torulopsis candida* and *C. guilliermondii*. The API 20C yeast system uses melezitose assimilation as the only reaction differentiating *C. tropicalis* from *C. albicans*; however, this reaction is not definitive since both organisms are variable in their ability to utilize melezitose (1). *Cryptococcus neoformans* and *C. tropicalis* cannot be differentiated if a false-negative inositol reaction occurs. The organisms with identical assimilation reactions can, however, be distinguished on the basis of their microscopic morphological features. Therefore, the API 20C system must be used in conjunction with microscopic morphology on cornmeal-Tween 80 agar. We also recommend that germ tube formation and chlamydospore production be used as additional criteria when *C. albicans* is differentiated from *C. tropicalis*.

The API system is not designed to provide a rapid presumptive identification of *C. neoformans* due to the slow growth of this organism. Such an identification may be obtained by use of previously evaluated rapid techniques including the rapid nitrate test (3), rapid urease test (7), and the caffeic acid-ferric citrate test (10). We feel that the final identification of *C. neoformans* should always be based on results of the nitrate test, carbohydrate assimilation tests, and pigment on niger seed agar.

Rapid, cost-effective identification of *C. albicans* is possible by use of the germ tube test. Therefore, inoculation of API strips with germ tube-positive organisms does not appear necessary. However, the API 20C should be useful in differentiating *C. stellatoidea* from *C. albicans* and in identifying germ tube-negative variants of *C. albicans*.

At present, *C. lambica* and *C. luteolus* are not included in the data base of the API 20C system.

However, the manufacturer stated that the assimilation profiles of these two organisms will be added to the system once a sufficient number of isolates have been obtained and characterized.

In conclusion, we feel that the modified API 20C is a commercial system useful for the identification of yeasts from clinical specimens. The system, although reliable, is not complete and is designed by the manufacturer to always be used in conjunction with microscopic morphological features for definitive identification. In addition, it is not designed for the rapid presumptive identification of such organisms as *C. neoformans*. Other biochemical tests may be used for this purpose. The modified API 20C system is technically less complex than the original, does not require any medium preparation in the laboratory, is stable at 4°C for 1 year from the date of manufacture, and requires little storage area. These features should increase the ability of even small laboratories to identify yeasts of medical importance. Acquisition and characterization of new and additional isolates by the manufacturer should result in improvement and expansion of the system.

ACKNOWLEDGMENTS

This work was supported in part by Analytab Products, Inc., Division of Ayherst Laboratories, Plainview, N.Y.

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